Supplemental Data

The linker histone H1.2 is a new component of the nucleolar organizer regions

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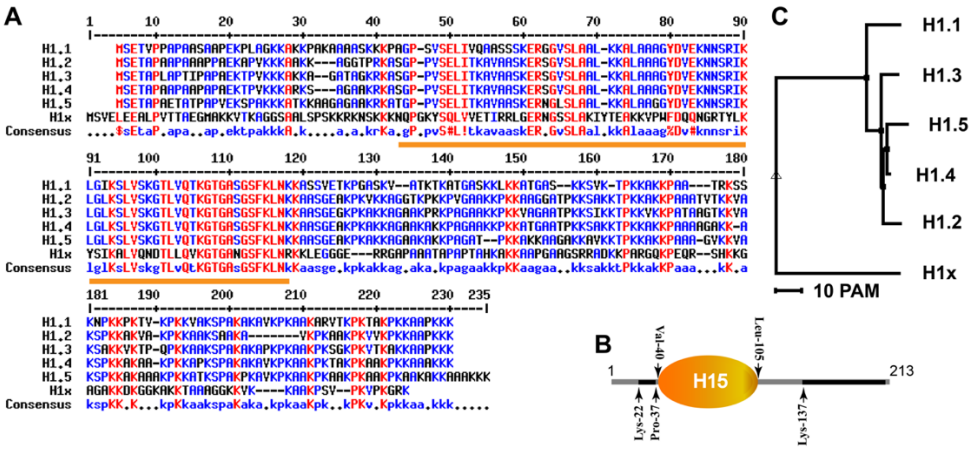
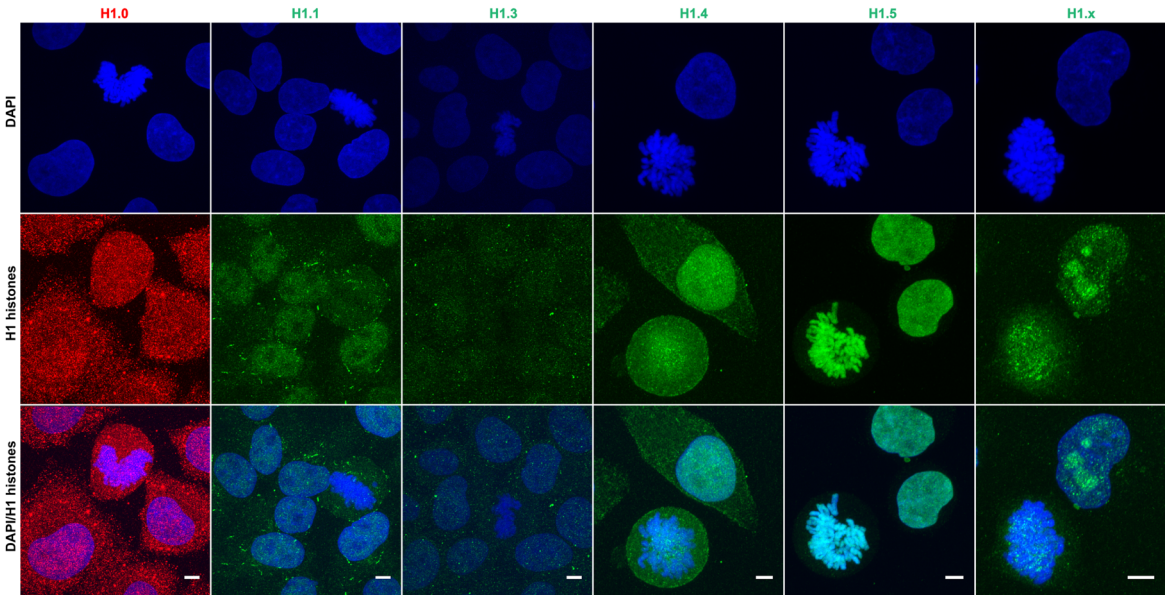
Supplemental Table S1

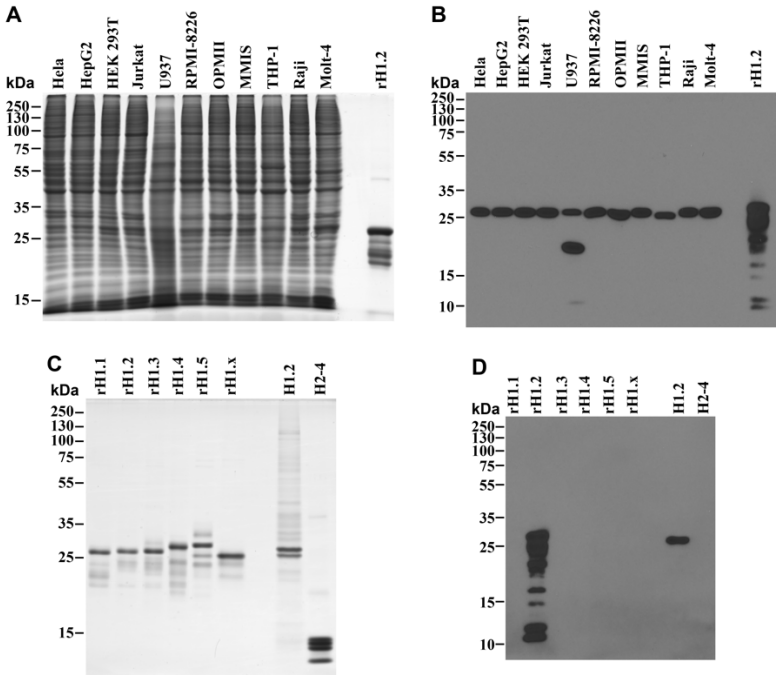
	LC-MS/MS analysis of affinity-isolated UBF-binding proteins					
Sample	**Protein name**	**Nucleolar Protein**	**Mw (kDa)**	**PIP (%)**	**EUP**	**PSC (%)**
Band-1	Pre-mRNA-processing-splicing factor 8 (PRPF8)	NO	27.4	96.9	1	0.3
	U5 small nuclear ribonucleoprotein 200 kDa helicase (SNRNP200)	NO	24.5	99.8	1	0.4
Band-2	Heterogeneous nuclear ribonucleoproten (HNRNPU)	NO	90.6	99.3	1	1.9
	U5 small nuclear ribonucleoprotein component (EFTUD2)	NO	109.4	100	2	2.7
Band-3	Nucleolin (NCL)	YES	76.6	100	23	31.4
Band-4	Nucleolin (NCL)	YES	76.6	100	10	17
	Nucleolar RNA helicase 2 (DDX21)	YES	87.4	99.8	2	2.6
Band-5	FACT complex subunit SSRP1 (SSRP1)	NO	81.1	98.2	1	1.1
	Nucleolar RNA helicase 2 (DDX21)	YES	87.4	98.4	1	1.2
	Nucleolin (NCL)	YES	76.6	99.9	1	2.0
Band-6	Nucleolin (NCL)	YES	76.6	100	18	23.2
	Heterogeneous nuclear ribonucleoprotein R (HNRNPR)	NO	70.9	100	6	10.6
	X-ray repair cross-complementing protein 6 (XRCC6)	NO	69.9	100	5	10.2
Band-7	Coiled-coil domain-containing protein 74B (CCDC74B)	NO	41.8	92.5	1	4.0
	Nucleophosmin-1 (NPM1)	YES	32.6	100	6	28.7
	Heterogeneous nuclear ribonucleoproteins C1/C2 (HNRNPC)	NO	32.3	100	4	16.4
Band-8	40S ribosomal protein S3a (Fragment) (RPS3A)	YES	22.1	100	4	18.4
	Histone H1.2 (HIST1H1C)	NO	21.4	100	7	18.8
	U1 small nuclear ribonucleoprotein A (SNRPA)	NO	31.3	100	4	15.2
Table legend	**Mw**, molecular weight; **PIP**, Protein identification probability**; EUP**, exclusive unique peptide count; **PSC**, percentage sequence coverage. **Nucleolar**, subcellular localization was based on the `Human Protein Atlas' (http://www.proteinatlas.org/)					



Supplemental Figure S1. Sequence alignment and homology between H1 histone variants. NCBI amino acid sequences for H1 histone variants H1.1 (NM_005325.3), H1.2 (NM_005319.3), H1.3 (NM_005320), H1.4 (NM_005321.2), H1.5 (NM_005322.2) and H1x (NM_006026.3) were aligned using the MultAlin software (http://multalin.toulouse.inra.fr/multalin/). *A*, detailed output sequence alignment in gif image format. Red, identical sequences. Blue, conserved sequences. A highly conserved globular domain (GD) across all H1 variants, which is flanked by a short N-terminal domain (NTD) and a long C-terminal domain (CTD), is underlined in orange. Amino acid positions are indicated on the top of the alignment. *B*, structure characteristics of histone H1.2. All these H1 variants have non-structured NTD and CTD but characterized by a structured middle GD known as H1/H5 (H15) globular domain (http://prosite.expasy.org/cgi-bin/prosite/nicedoc.pl?PS51504). In the two non-structured regions, those highlighted in `black' are predicted to bind to DNA and/or RNA (https://www.predictprotein.org/visual_results?req_id=583561). Arrows indicate the boundaries of domains. DNA-binding sites have been predicted in both NTD and CTD. RNA-binding regions are mostly predicted in CTD. *C*, phylogenetic tree presentation of the aligned H1 variant sequences to indicate their inter-relatedness in PAM distance.



Supplemental Figure S3. Different subcellular localization of H1 vaiants. HeLa cells were fixed and permeabilized. Cells were first stained for 1 hr with specific anti-H1 antibodies (Abcam): mouse anti-H1.0 (ab11079) and rabbit anti-H1.1 (ab17584), anti-H1.3 (ab183736), anti-1.4 (ab105522), anti-1.5 (ab18208), and anti-H1.x (ab31972). After washing, cells that were incubated with the mouse anti-H1.0 antibody, were incubated with goat anti-mouse IgG (Cy3, red). Cells that were incubated with the rabbit anti-H1 antibodies, were incubated with goat anti-rabbit IgG (AF488, green). After mounting with DAPI-containing medium, cells were analyzed by confocal microscopy. Scale bars, 5 μm.



Supplemental Figure S3. Specificity of the anti-H1.2 antibody and its reactivity with endogenous and recombinant H1.2 proteins. *A*, protein profiles of 11 different human cell lysates by SDS-PAGE and Coomassie blue staining. Note protein degradation in the U937 cell lysate. Recombinant H1.2 was included as a positive control. *B*, Western blotting of the 11 cell lysates shown in (*A*). Note that the antibody reacted with a 27-kDa protein, the size of full-length H1.2, in all cells. Additional proteins that reacted with the antibody was only detected in the U937 cell lysate which were smaller and could be H1.2 fragments. *C*, six recombinant H1 variants (rH1.1-rH1.5, rH1.x), partially purified endogenous H1 from HeLa cells, and purified endogenous core histones (H2A, H2B, H3 and H4) were examined by SDS-PAGE and Commassie blue staining. cDNA for these six H1 variants were PCR-amplified using the following primers and cloned into the NdeI and BamHI sites of the pET28 vector:
H1.1 (5'-agccatatgatgtctgaaacagtgcctcccg-3'/5'- ttcggatccgagccgttgggttactagaagaaac-3'),
H1.2 (5'-atggctagcatgtccgagactgctcctgcc-3'/5'-ttcggatccgggttttagaagtaggcgttcgc-3'),
H1.3 (5'-atggctagcatgtcggagactgctccacttg-3'/5'-ttcggatccgaacgtcccgccagtttcac-3')
H1.4 (5'-atggctagcatgtccgagactgcgcctgccg-3'/5'-ttcggatccgggcttctaagcagttggccaaagg-3')
H1.5 (5'-atggctagcatgtcggaaaccgctcctgccgag-3'/5'-ttcggatccgttgcggttttcacacgccagcttc-3')
H1.x (5'-atggctagcatgtccgtggagctcgaggaggc-3'/5'-ttcggatcctcacttgcggcccttgggcactttg-3')
To different extents, degradation was persistently detected before bacteria were lysed for purification as was also observed previously (1). *D*, by Western blotting, the anti-H1.2 antibody reacted with rH1.2 and purified H1 but showed no reactivity with purified core histones or other H1.2 variants.

References

1. Konishi, A., Shimizu, S., Hirota, J., Takao, T., Fan, Y., Matsuoka, Y., Zhang, L., Yoneda, Y., Fujii, Y., Skoultchi, A. I., and Tsujimoto, Y. (2003) Involvement of histone H1.2 in apoptosis induced by DNA double-strand breaks. *Cell* **114**, 673-688